Genetic integrity of the European grayling (*Thymallus thymallus*) populations within the Vienne River drainage basin after five decades of stockings

by

Henri PERSAT* (1), Karin MATTERSDORFER (2), Stéphanie CHARLAT (3),
Tamara SCHENEKAR (2) & Steven WEISS (2)

© SFI

Received: 2 Jul. 2015
Accepted: 16 Dec. 2015
Editor: K. Rousseau

Key words
Salmonidae
Thymallus thymallus
Loire
Genetic diversity
Stocking
Survival

Abstract. – The upper Vienne River is the westernmost drainage in the natural distribution of European grayling *Thymallus thymallus* (Linnaeus, 1758). The grayling extension across this sub-basin of the Loire catchment has been dramatically reduced through habitat degradation, which began in the 19th century with the construction of milldams and eventually large hydroelectric dams, which flooded former grayling habitat (i.e. fast flowing waters) under increasingly larger reservoirs. Throughout the 20th century, habitat degradation, fragmentation and pollution have strongly affected the remaining isolated populations. Since the 1960s, fishing authorities have attempted to compensate for these declines with stocking programs. Most stocked fish stem from imported eggs or fry of unknown origin. However, the efficacy of this policy has never been assessed. Here, we aim to assess the genetic traces of these stocking programs as an indirect measure of the long-term survival of stocked fish. Sampled grayling from four localities in the Upper Vienne basin were analysed at both mtDNA (N = 37, control region) and nDNA levels (N = 88, 10 microsatellites), and compared to populations representative of surrounding drainage basins or fish farms. Microsatellite analysis demonstrated low genetic diversity within samples, typical for the Loire drainage. Both Factorial Correspondence and “Structure” analyses presented Upper Vienne grayling as a comprehensive genetic unit distinct from diverse samples from the Rhine, Rhone and Danube basins, as well as other regions of the Loire catchment and the sampled hatcheries. Within the Vienne, only the uppermost sample differed from the other sites. Among the 37 individuals sequenced from the Upper Vienne, only three control region haplotypes were identified, all closely related to other haplotypes observed in Loire and Allier (a Loire tributary) rivers. These results strongly support that grayling in the Upper Vienne drainage represent native genetic stocks with no signs of non-native introgression. The only evidence potentially supporting a stocking contribution is the co-occurrence of a haplotype in the Allier catchment and Vienne River, although we cannot exclude that this haplotype is also native to both sites. These results suggest that five decades of stocking have provided no added reproductive value to the Vienne populations. We thus hypothesize that long-term survival of stocked fish has either been low or non-existent.

Résumé. – Intégrité génétique des populations d’ombre commun (*Thymallus thymallus*) du bassin de la Vienne après cinq décennies d’alevinages.

Le bassin amont de la Vienne constitue la limite occidentale de l’aire de répartition naturelle de l’ombre commun *Thymallus thymallus* (Linnaeus, 1758) en Europe, mais depuis le XIXe siècle la construction de multiples moulins puis de grands barrages hydroélectriques a singulièrement réduit et fragmenté le linéaire de cours d’eau habitable par cette espèce rhéophile. De plus, les populations subsistantes ont été exposées aux pollutions industrielles des XIXe et XXe siècles, ce qui a conduit à l’extinction de beaucoup d’entre elles. Pour tenter de compenser ce déclin, dès le milieu des années soixante les autorités locales de la pêche ont procédé régulièrement à des déversements d’alevins. Ces derniers ont été fournis par divers pisciculteurs publics ou privés important leurs œufs de l’extérieur, mais sans fournir d’indications précises quant à leur origine exacte. Par ailleurs, l’efficacité de ces alevinages n’a jamais été réellement évaluée. Le but de cette étude était donc d’examiner l’impact génétique de ces repeuplements. Quatre populations du bassin de la Vienne ont été analysées à la fois au niveau de l’ADN nucléaire (10 microsatellites) et de l’ADN mitochondrial (région de contrôle) et comparées à des populations représentatives des bassins limitrophes ou des productions de piscicultures. Le séquençage de l’ADN mitochondrial de 37 individus “sauvages” n’a fourni que trois haplotypes, ce qui est peu mais comparable à ce qui est observé dans le bassin de la Loire et aussi souvent ailleurs. Ces trois haplotypes sont clairement apparentés aux haplotypes observés dans les bassins de l’Allier et de la Loire en Auvergne, et par conséquent éloignés de tous les autres haplotypes connus jusqu’à présent en Europe. Par ailleurs, deux de ces haplotypes semblent spécifiques du bassin de la Vienne tandis que le troisième, observé seulement dans la population la plus aval de la Vienne, était déjà connu du haut Allier. L’analyse des microsatellites de 88 individus “sauvages” a également fait apparaître une faible diversité dans toutes les populations, mais comparable à celle des autres populations ligériennes. Que ce soit par Analyse Factorielle des Correspondances ou par Analyse “Structure”, les populations de la haute Vienne forment un ensemble homogène bien distinct de toutes les autres populations considérées. Tout

---

(1) Laboratoire d’Écologie des Hydrosystèmes Naturels et Anthropisés UMR CNRS 5023, Bâtiment Forel, Université Claude Bernard Lyon 1, 69622 Villeurbanne, France.

(2) University of Graz, Institute of Zoology, Universitätsplatz 2, A-8010 Graz, Austria. [steven.weiss@uni-graz.at].

(3) Fédération de la Haute-Vienne pour la Pêche et la Protection du Milieu Aquatique, 31 rue Jules Noël, 87000 Limoges, France. [stephanie.charlat@federation-peche87.com]

* Corresponding author [henri.persat@univ-lyon1.fr]

Cybium 2016, 40(1): 7-20.

au plus la population amont de la Vienne sort-elle un peu du lot. Ces résultats prouvent que les populations de la haute Vienne sont bien natives de ce bassin, même si elles s'apparentent aux autres populations naturelles du bassin de la Loire. Le seul indice d'une possible trace génétique d'alevinages antérieurs réside dans la présence chez quelques poissons de la Vienne aval d'un haplotype commun au haut Allier, mais on ne peut exclure que sa présence en Vienne y soit naturelle. L'absence d'autres traces de contributions génétiques allochtones à la Vienne suggère que cinq décennies d'alevinages en Ombres n'ont en rien contribué au maintien des populations locales. La survie ou le succès reproducteur des poissons déversés semblent avoir été nuls, quels que soient la souche ou le stade de développement employés. La présence d'une population autochtone semble un facteur déterminant dans cet échec puisque de nombreux exemples d'implantations réussies sont à signaler dans des cours d'eau où l'espèce était absente auparavant.

For well over half a century, the viability and efficacy of stocking hatchery-reared salmonids into freshwaters have been a topic of both extensive research and controversy (Miller 1953, 1958; Vincent, 1987; Weiss and Schmutz, 1999 and references therein). Despite several reviews on the topic (e.g. Cresswell, 1981; Finnegan and Stevens, 2008; Hansen *et al.*, 2009), generalizations on the overall success of stocking (from a management perspective) or the extent of its negative consequences have been elusive. For brown trout for instance, Näslund (1998) reported experimental survival rates between 0 and 26% for 0+, 1+ and 2+ old stocked fish after 12 months across several Swedish streams, and Weiss and Schmutz (1999) reported similarly disparate rates (<1% to 19%) for 2+ and 3+ old hatchery-reared fish in two Austrian streams, compared to much higher survival for their wild counterparts. Champigneulle *et al*. (2002) pointed out that "Atlantic" brown trout introduced as fingerlings in the Doubs River suffered a higher angling pressure than the native "Mediterranean" trout during the following years. As a result, larger trout found in the population were mostly "Mediterranean". This trend might contribute to the genetic segregation observed in that river by Largiadèr and Scholl (1996). Harbicht *et al*. (2014), in a meta-analysis of 37 comparisons of survival between wild, hybridized and hatchery-reared salmonids, concluded that wild fish almost always exhibit higher survival rates than hybridized or hatchery-

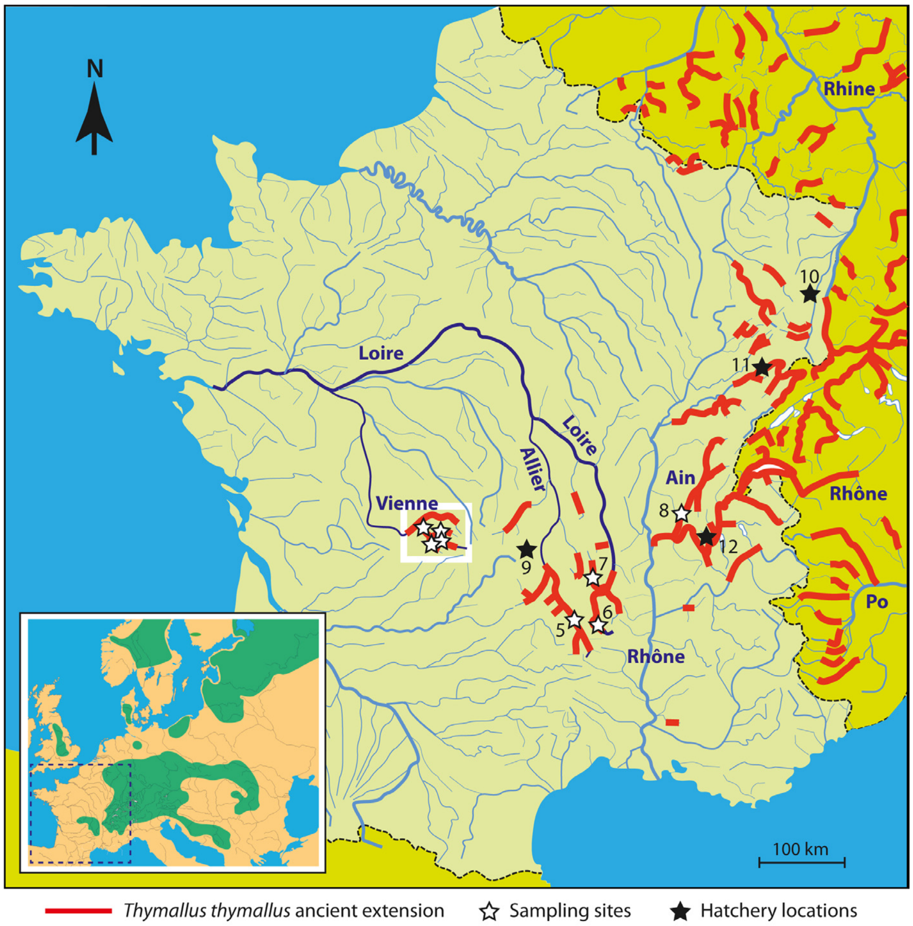

Figure 1. - Distribution of grayling in Europe (green) and its former distribution in France around 1900 (red). Sample sites (white stars = wild populations, black stars = hatcheries) are numbered following table II.

reared fish. Additional to estimating survival of hatchery-reared fish, the degree of introgression has been investigated across many different salmonid fishes. For instance, some authors suggest that the intensity of stocking correlates with non-native introgression (Marie *et al.,* 2010), while others invoke environmental integrity as a potential barrier for introgression (Seehausen *et al.*, 2008; Bittner *et al.,* 2010; Winkler *et al.,* 2011), as well as various biological isolating mechanisms (Winkler *et al.,* 2011). In any case, the rationality of stocking programs as a means of compensating (or mitigating) pervasive habitat changes primarily caused by human activity remains questionable, especially in flowing waters.

Studies on the success or effects of stocking European grayling *Thymallus thymallus* (L., 1758) are much less numerous as those for salmon and trout (but see Carlstein, 1997; Turek *et al.*, 2010), but presumably the efficacy of stocking for this species must be at least as problematic as it is for other salmonid species. European grayling is considered a sensitive salmonid confined to the large, clean, cold, and fast running rivers of North and Central Europe (Fig. 1; Persat *et al.,* 1978). Its requirements are so specific that Huet (1949) created a specific zone for it in his river zonation scheme.

Nonetheless, and in spite of its increasing popularity as a sport fish, grayling populations have suffered serious declines, especially in Central Europe and along the southern limits of its distribution (see Weiss *et al.* (2013) and reference therein; Persat (1996) for France). The causes of these declines are considered to be multi-factorial and include various river engineering activities, increased predation pressure by cormorants *Phalacrocorax carbo* (L., 1758), interspecific competition with introduced salmonids such as rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and climate change (Uiblein *et al.*, 2001). Due to the small size of their larvae, rearing grayling is generally considered more difficult than trout, charr and salmon, and thus grayling hatcheries in France, at least initially, rarely relied on rearing local strains, preferring imported eggs or fry from longer established sources in Central and Northern Europe, usually centred near large natural spawning grounds. Thus, commercially available grayling strains have been quite diverse across time and space compared to, for example, brown and rainbow trout, which often stem from centralized industrial production facilities. Furthermore, extensive genetic investigations on European grayling over the last 15-20 years clearly demonstrated that their natural genetic diversity, especially at the lineage or basin level, far exceeds that of other European salmonids (Sušnik *et al.*, 1999; Gross *et al.,* 2001; Weiss *et al.,* 2002, 2013; Gum *et al.,* 2005, 2009). Some of these investigations have clearly highlighted cross-basin introgression due to stocking activities, and a recent report covering regions of Northern Italy and Western Austria even has demonstrated a nearly complete local displacement of native stocks with hatchery strains (Meraner *et al.,* 2014; Weiss *et al.*, 2015). However, this same report highlighted nearly pure or pure genetic stocks in some systems where frequent management activities have taken place. This supports, for example, the observations of Meraner *et al.* (2013) in the Upper Drau River in Austria, where a purely native sample of grayling was reported, despite the fact that the whole river has been extensively managed with mixed and foreign stocks of fish. Thus, there is no clear consensus on the long-term survival of stocked grayling, or on the levels of genetic introgression observed in grayling populations that have been stocked with foreign strains.

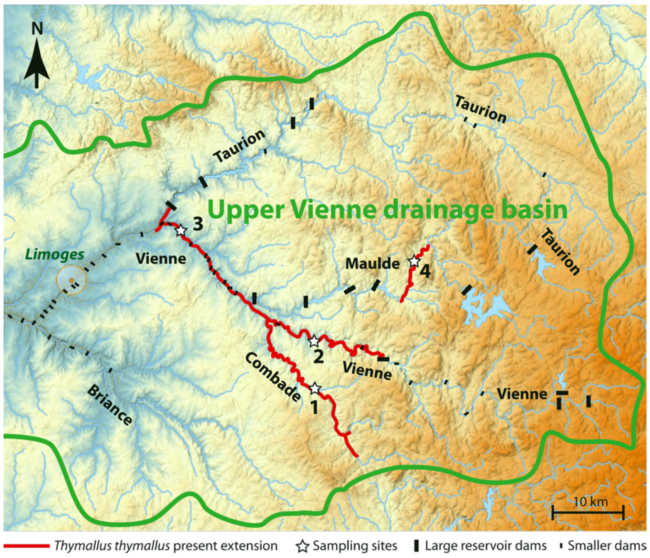

Figure 2. - Close-up of grayling distribution and four sampled sites (stars) within the upper Vienne district in 2012 (relief background from IGN-Geoportail).

In France, stocking of grayling started slowly in 1960s with eggs imported mainly from Central Europe, a practice that became more common after 1970. A progressive shift to local production of eggs from fish matured in captivity took place after 1980, and was mainly driven by fishing authorities (Conseil Supérieur de la Pêche, and Angling Federations; Carmié *et al.*, 1985). However, the fickle nature of the production limited these activities, and private fish farms using imported eggs still provide a significant amount of the fry stocked into French rivers. A first genetic-based evaluation of the potential contribution of stocking within the partitioned river network of the French Upper-Rhone River was attempted by Persat and Eppe (1997), using enzymatic markers. However, this effort primarily showed a significant difference between the main channel and tributaries, but this difference was not due to the local hatchery: indeed, almost no individuals from these regular stockings were found.

Ongoing investigations on the genetic diversity of the native grayling populations in France using mtDNA and microsatellite markers confirmed significant divergence between the three major drainages (Rhine, Rhone and Loire) and at a finer level, within each drainage according to their size and tributary complexity (Cattaneo *et al*., 2011; Persat and Weiss, unpubl. data). Thus, the natural genetic structure of grayling in France makes it rather easy to distinguish native versus non-native genotypic profiles. This is especially true for the Loire drainage (Fig. 1), as it is naturally inhabited by one of the most genetically divergent lineages in Europe (Weiss *et al.,* 2002).

Table I. - Amount of grayling stocked per year in the Upper Vienne drainage basin (mostly with fry before 1990, mainly with fingerlings after 1990).

Year	Amount	Year	Amount	Year	Amount
1966	10,000	1982	98,000	1998	?
1967	30,000	1983	85,000	1999	ca. 6,000
1968	40,000	1984	76,000	2000	ca. 4,500
1969	20,000	1985	74,000	2001	ca. 26,000
1970	60,000	1986	55,200	2002	16,000
1971	65,000	1987	43,000	2003	9,000
1972	120,000	1988	94,000	2004	20,000
1973	64,000	1989	?	2005	22,000
1974	100,000	1990	?	2006	14,000
1975	31,200	1991	?	2007	17,000
1976	40,000	1992	?	2008	6,300
1977	77,000	1993	?	2009	12,000
1978	55,000	1994	12,400	2010	20,000
1979	67,000	1995	7,000	2011	10,000
1980	110,920	1996	ca. 2000	2012	10,000
1981	93,500	1997	?		

In order to test the genetic impact of stocking on *Thymallus thymallus*, we selected populations of the remote Upper Vienne River sub-basin, which is isolated from the other native populations of the Loire-Allier sub-basin by approximately 800 km of riverine distance. Moreover, the Upper Vienne is under jurisdictional control of a single angling federation, which was able to provide us an overview of the stocking operations performed over the last 50 years (Tab. I). The fish were provided by more than five different public or private fish farms across time, four of which we have sampled within this study. These sources are not exhaustive, as local angler societies may have contributed stocked fish on their own initiative. Mostly, the origin of the fish was unrecorded and differed across time and facilities. We gathered samples from four farms that still currently producing grayling to represent gene pools of potential relevance to historical stocking, whereby only one (Besse farm) has been officially involved in recent stockings into the Vienne basin.

MATERIAL AND METHODS

Samples from a total of 384 *T. thymallus* individuals from 13 locations, with a focus on the Loire basin, were included in the study (Figs 1, 2; Tab. II). Samples included seven locations in the Loire catchment (four of which come from the Upper Vienne), four fish farms, and two reference populations representing the Rhone drainage and the northern alpine Danube lineage (see Weiss *et al.,* 2002 for an overview of major genetic lineages). Several individuals from the Ance du Nord River in the Loire basin were additionally included (for mtDNA analysis only) as reference material. We also included a Finnish sequence because Finnish eggs were sometimes imported by local farms.

Table II. - Samples used in this study, including the name of the population, the major drainage, geographic coordinates and the number of individuals typed for either microsatellites or the complete mtDNA control region.

Population	Drainage	Pop. Code	Coordinates	*N* msats	*N* mtDNA
Vienne					
Combade	Loire	1	1°37’40”E -45°42’47”N	37	11
Vienne upstream	Loire	2	1°37’22”E-45°45’45”N	34	11
Vienne downstream	Loire	3	1°25’08”E-45°52’55”N	15	13
Maulde	Loire	4	1°46’22”E-45°50’35”N	2	2
Allier (Vabres)	Loire	5	3°40’26”E -44°55’05”N	41	10
Upper Loire	Loire	6	3°55’30”E-44°51’54”N	43	19
Ance du Nord	Loire	7	4°01’52”E-45°15’44”N	–	7
Ain (Mollon)	Rhone	8	5°14’52”E-45°57’20”N	42	17
Besse	*Hatchery*	9	2°55’25”E-45°30’55”N	29	11
Obenheim	*Hatchery*	10	7°41’16”E-48°21’20”N	40	11
Chauvey (Appenans)	*Hatchery*	11	6°33’58”E-47°26’48”N	33	7
Chazey-Bons	*Hatchery*	12	5°40’00”E-45°48’42”N	43	10
Kohlenbach	Danube		12°23’00”E-47°39’56”N	25	15
Total				384	144

For the Vienne basin, sampling was done by electric fishing in Nov. 2011. The highest apparent density (individuals collected per hour) was found in the Combade River, whereas the lowest density (two individuals collected over several hundred meters) was recorded in the Maulde River in the only short river reach still inhabited by grayling between two large dams (Fig. 2). Fish were aged based on scale analysis (most fish were 1+ or 2+ old, i.e. from 2010 or 2009 generations) and each fish > 20 cm was photographed. Small fin-clips were taken from the tip of the pelvic fin and stored in 96% ethanol before releasing the fish unharmed into the river.

Fish farm samples were generously provided by angling societies, and all represent potential source material for past or present stocking programs in the Upper Vienne basin. These are not necessarily representative of all facilities that have produced grayling from imported eggs, as many facilities no longer exist. The samples also represent "batches" and may not fully cover the genetic diversity of each facility – grayling egg batches in such farms are routinely separated from one another, to avoid mixing healthy and unhealthy broods (Carmié *et al.,* 1985) and thus different tanks may represent different gene pools. Three of the farms claim to produce their brood from local stocks; i.e. the Rhine River (Obenheim fish farm, Bas-Rhin Federation), the Allier River (Besse-en-Chandesse, Puy-de-Dôme Federation) and the Rhone River (Chazey-Bons, Ain Federation). The fourth farm (Appenans, Chauvey) is a private facility mostly dependent on imported eggs, though some production is claimed to stem from local Doubs (Rhone drainage) stocks.

Genomic DNA was isolated from preserved tissue using a high salt (ammonium acetate) extraction protocol, modified from Miller *et al.* (1988). A total of 12 published microsatellite loci were combined into two multiplex reactions as listed in table III and applied to the screening of all 384 samples. PCR conditions were 5-30 ng template DNA, 2.5 μl 2x Type-it Multiplex Master Mix (Qiagen), 0.2 μM of each primer in a final reaction volume of 5 μl. The PCR cycles consisted of an initial denaturation step of 95°C for 5 min followed by 25 cycles of denaturation at 95°C for 30 sec, annealing at 59°C (7-plex) or 60°C (5-plex) for 1 min 30 sec and extension at 72°C for 30 sec and a final extension step at 60°C for 30 min. The alleles were scored using an internal size standard (GeneScan-500 ROX, Applied Biosystems) and analyzed with GeneMapper® software version 3.7 (ABI).

Observed and expected heterozygosity, the mean number of alleles and deviations from Hardy-Weinberg Equilibrium (HWE) (using the intrapopulation fixation index F_{IS}) were calculated using GENETIX version 4.05 (Belkhir *et al.,* 1996). Statistical significance was obtained with 10 000 permutations and a table-wide Bonferroni correction. To display genetic relationships among individuals we carried out a Fac-

Table III. - Microsatellite locus description including the arrangement of the 7-plex and 5-plex reactions, the locus name and repeat motif, the primer sequence, the fluorescent-labelling dye, annealing temperature, original reference and focal species used to clone the locus. Note that the published names of Thy1 (BFRO004), Thy62 (BFRO010), and Thy54 (BFRO018) differ from those given here.

	Locus	Repeat motif	Primer sequence (5´-3´)	Dye	AT	Reference	Focal species
7 - Plex	Tth447	(TG)$_{17}$	F: CTTGATTGCCATTGGATTGT R: GTTTCTTCAACATCCTTGTCGCCTCTA	HEX	59°C	Junge *et al.* (2010)	*T. thymallus*
	Tth305	(CTTT)$_{8}$	F: CTTTGAATATGATGCGTGAAC R: GTTTCTTGAGTATACTGCAGATAGACCA	NED	59°C	Junge *et al.* (2010)	*T. thymallus*
	Thy1	(CA)$_{17}$	F: CGCATCTGTATGAAAAACCT R: TGGTTTGGTAGGAGTTTCGT	FAM	59°C	Sušnik *et al.* (1999a)	*T. thymallus*
	Tar101	(CTTT)$_{22}$	F: CAGAGCACACCAAGCAGAG R: AGGGCAAGTCATTCCAGTC	HEX	59°C	Diggs and Ardren (2008)	*T. arcticus*
	Thy62	(AC)$_{17}$	F: GGA CGG AGC CAG CAT CAC R: GTTTCTTGATTTCATAATCAGGTCAATAGTCAT	NED	59°C	Sušnik *et al.* (2000)	*T. thymallus*
	Tar106	(CATA)$_{24}$	F: CGTCCAGTCTGACACAAAG R: GTTTCTTATGAACCGAAGGAATCATG	FAM	59°C	Diggs and Ardren (2008)	*T. arcticus*
	Tth445	(GATA)$_{20}$	F: TGA CGG CTA CAG GAA TTGT R: GTTTCTTCCACAGAGGGTTCTACATTG	NED	59°C	Junge *et al.* (2010)	*T. thymallus*
5 -Plex	Tar104	(CATA)$_{23}$	F: TCTTCTCAGTGGCATGACATC R: CCTCGTACTCTCTCTTGTCCC	NED	60°C	Diggs and Ardren (2008)	*T. arcticus*
	Thy54	(TG)$_{13}$	F: AGAGGGGTCCAGCAACATCA R: GTTTGGGGAACCAGTCTAAAGCCT	HEX	60°C	Sušnik *et al.* (1999b)	*T. thymallus*
	Tar213	(CTTT)$_{33}$	F: TTTCCACAGAGGGTTCTACAT R: GTTTCTTACTAGAGCAGGGCAGCAGA	HEX	60°C	Diggs and Ardren (2008)	*T. arcticus*
	Tar414	(AGAT)$_{21}$	F: GTCGGGACATGGACTCTACA R: GTTTCTTGCAATGCCTCTTTATAGCTT	NED	60°C	Diggs and Ardren (2008)	*T. arcticus*
	Tth446	(ATCT)$_{15}$	F: GCCATTCACCCATACTATGC R: GTTTCTTCCATTCAGCCACTAGAGC	FAM	60°C	Junge *et al.* (2010)	*T. thymallus*

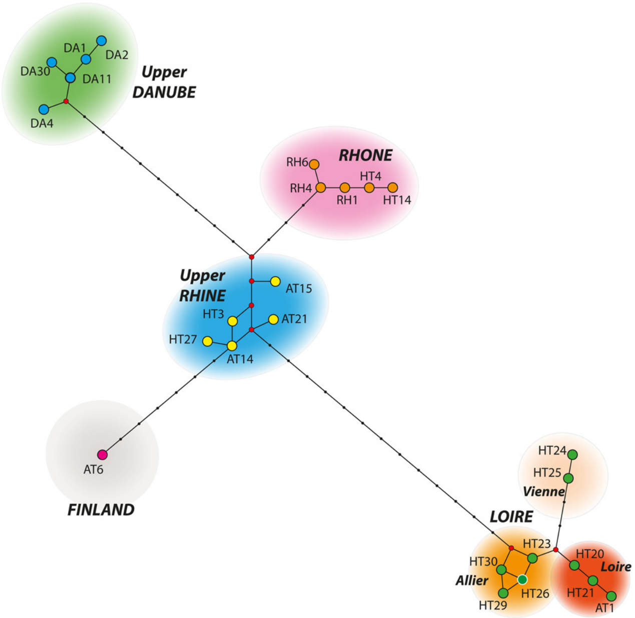

Figure 3. - Median Joining network of haplotypes found in this study augmented by additional published haplotypes provided for reference. All new haplotypes use the simple abbreviation "Ht" with a serial number reflecting the order in which they were first found in our data. The small black dots indicate substitutional steps whereas the small red dots represent multifurcating nodes. The following haplotypes and corresponding clade names stem from Weiss *et al*. (2002). "*Danube drainage (Northern Alps)*": Da1, Da2, Da4, Da11; "*Mixed Central Europe*": Rh1, Rh4, Rh6 all first reported from the Rhone basin; "*Mixed Central Europe*": At14, At15 both frequent and widespread in the Rhine basin; "*Scandinavia*": At6 reported from Finland; and AT1 first reported from the Loire River.

torial Correspondence Analysis (FCA) with the GENETIX software package. Differences among samples are based on the presence and absence of alleles, and are displayed in two two-dimensional scatter plots using the first four vectors. To determine the number of genetically homogenous groups of individuals (i.e. clusters) and estimate potential introgression of hatchery strains into wild populations we used the Bayesian assignment procedure implemented in the program Structure v.2.3.3 (Pritchard *et al*., 2000). The procedure assigns individuals according to their genotypes at multiple loci into *K* clusters without using any *a priori* population information. We ran Structure using the admixture-model with correlated allele frequencies (Falush *et al*., 2003). A Markov chain Monte Carlo (MCMC) sampling procedure with 200,000 iterations after a burn-in of 50,000 iterations was used for estimating posterior probabilities. We carried out ten replicate runs for each estimation of *K* ranging from 1 to 14. We used the method of Evanno *et al*. (2005), implemented in the program Structure Harvester v.0.6.6 (Earl and von Holdt, 2012), to determine the most likely value of *K* (http://taylor0.biology.ucla.edu/struct_harvest/).

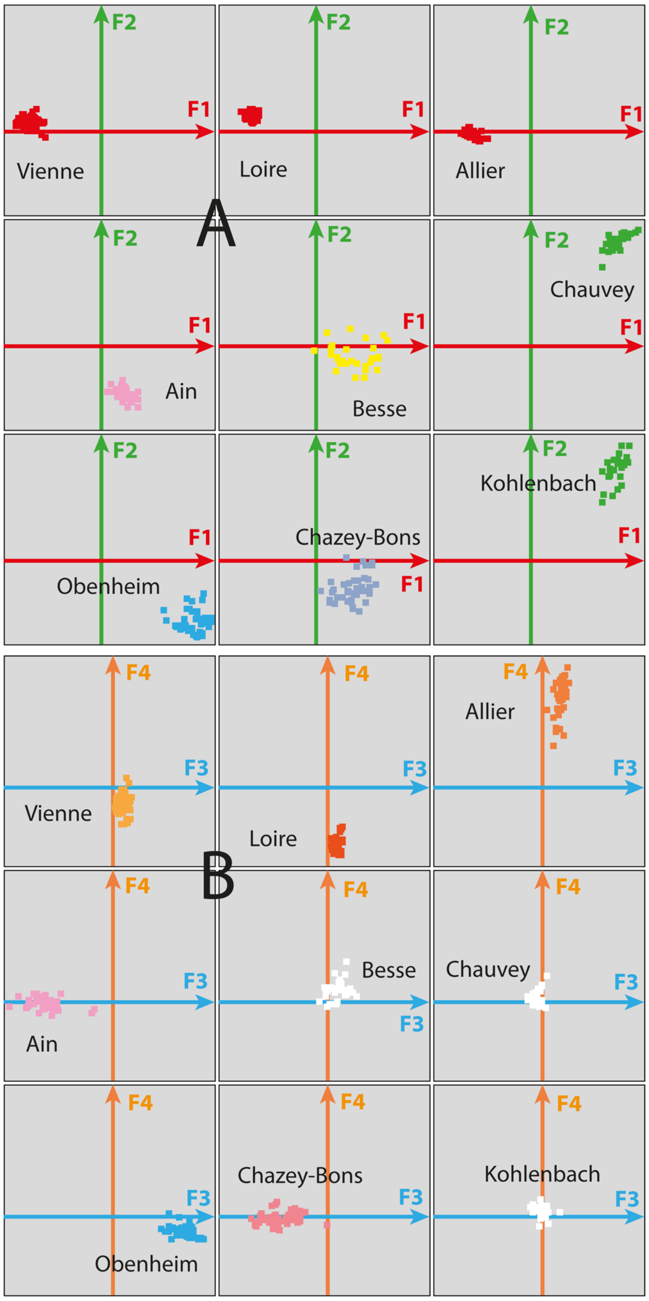

Figure 4. - Factorial Correspondence Analyses (FCA) of individuals based on the presence and absence of microsatellite alleles. **A**: Bi-variate plot of the first two FCA factors (F1 & F2), graphically partitioned by population. **B**: Bi-variate plot of the third and fourth FCA factors (F3 & F4) partitioned by population. The percentage inertia for each factor is as follows: FC1 = 4.44%; FC2 = 4.03%; FC3 = 3.46%; FC4 = 2.82%.

mtDNA analysis

The complete mtDNA control region (CR) was amplified in 144 individuals (including 34 used in previous reports) using the primers LRBT-25 (Forward, 5'-AGA GCG CCG GTG TTG TAA TC-3') and LRBT-1195 (reverse, 5'-GCT

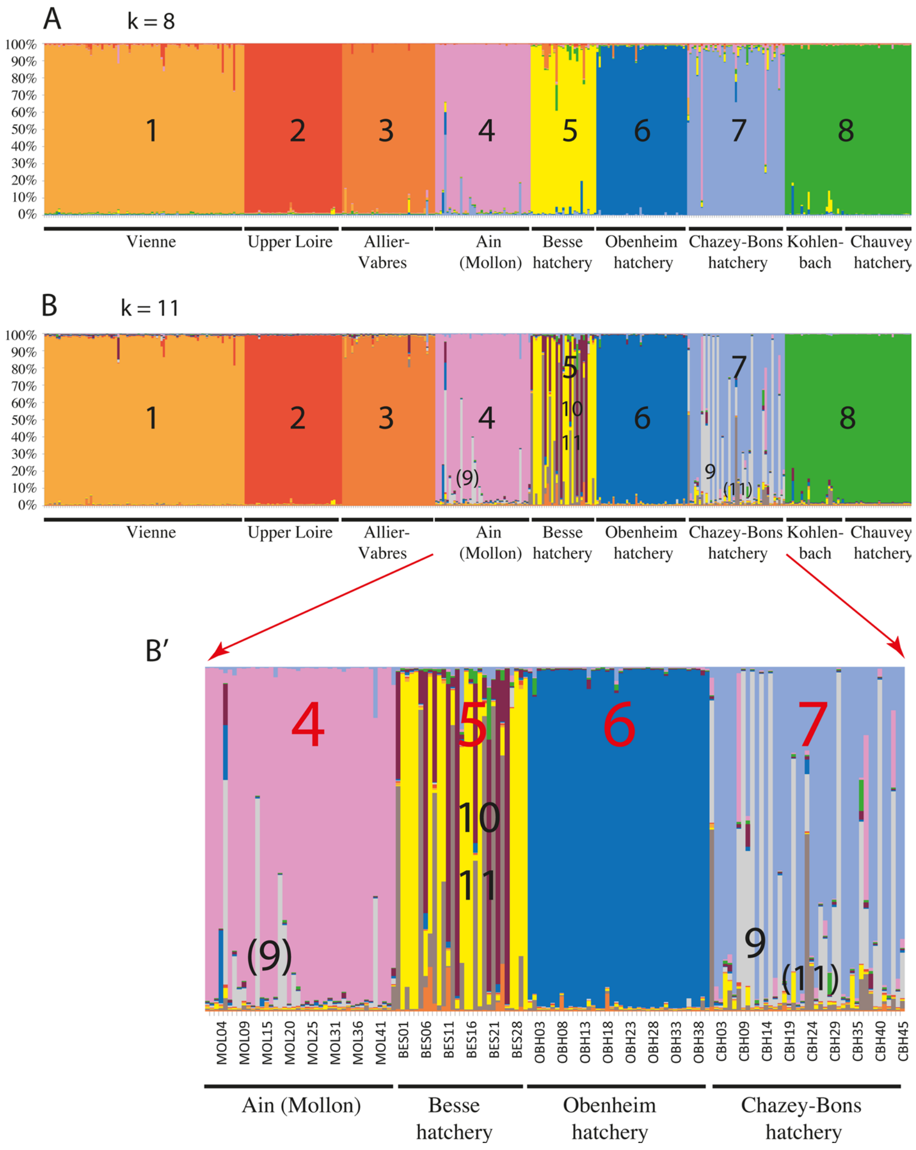

Figure 5 - Results of the Structure analysis (Q-values) for 8 loci (A) and 10 loci (B). Colours represent the different "k" units chosen using the approach of Evanno *et al.* (2005). Population names are shown on the x-axis, and Q-values (contribution or assignment from each k partition) are shown on the Y-axis. For 8 loci, k = 8, and for 10 loci k = 11.

AGC GGG ACT TTC TAG GGT C-3'; Uiblein *et al.*, 2001). Amplification was performed with a total volume of 10 μl per sample, each consisting of 1 μl diluted DNA extract, 2 μl 5x Phusion® HF Reaction Buffer, 0.2 μl dNTPs (10 μM), 0.5 μl of each primer (10 μM.), 0.25 μl Phusion® High Fidelity Polymerase (2 units/μl) and 5.55 μl of deionized water. The amplification temperature profile was as follows: after an initial denaturation at 98°C for 30 sec, 35 cycles of 10 sec at 98°C, 30 sec at 55°C and 30 sec at 72°C were conducted followed by a final elongation step at 72°C for 10 min and a final hold at 8°C. The sequencing reaction was carried out with the internal primers D-Loop-Int5' (5'-ATA TAA GAG AAC GCC CGG CT-3') for the first part of the 5' end, CR-Int-3F-thy (5'- GAA ACC ACT CAC TGA AAG CCG-3') for middle of the PCR product, and CRII-Int-2F-thy (5'-GGA ATC CCC CGG CTT CTA C-3') for the 3' end. After sequencing, DNA fragments were purified with SephadexTM G-50 (Amersham Biosciences) following the manufacturer's instructions and visualized on a 3130 XL capillary sequencer (Applied Biosystems). Sequences were aligned visually using MEGA software v.5 (Tamura *et al.*, 2007). Subsequent to alignment, the poly-T region (see Sušnik *et al.*, 2006) was removed, leaving a total of 1048 bp for analysis. A median joining haplotype network was constructed using the program PopArt (http://popart.otago.ac.nz).

RESULTS

Two of the twelve microsatellites (Tth445 and Tth213) were removed from the analysis due to lack of polymorphism and problems with null alleles. The number of indi-

Table IV. - Occurrence of the different haplotypes in the populations of the Upper Vienne basin, in three other populations of the Loire drainage basin, in the Kohlenbach (Danube basin), in the Ain River (Rhone basin), and in four fish farms (in Italic).

	Haplotypes																			
	Loire basin									Danube basin				Rhone basin				Rhine basin		Total
	Vienne		?	Allier			Loire													
Population	Ht24	Ht25	Ht26	Ht29	Ht30	Ht23	Ht20	Ht21	At1	Da1	Da4	Da11	Da30	Rh1	Rh4	Ht4	Ht14	Ht3	Ht27	
Combade	9	2																		11
Vienne upstream	11																			11
Vienne downstream	9		4																	13
Maulde	2																			2
Allier Vabres			3	4	3															10
Upper Loire									19											19
Ance du N.							6	1												7
Ain aval														12	1	3	1			17
Besse						*4*						*7*								*11*
Obenheim																		*3*	*8*	*11*
Chauvey										*5*	*1*		*1*							*7*
Chazey-Bons														*1*		*9*				*10*
Kohlenbach										12	1	2								15
Total	31	2	7	4	3	4	6	1	19	17	2	9	1	13	1	12	1	3	8	

viduals successfully processed for the remaining 10 microsatellites or mtDNA is listed in table II. For mtDNA, 37 of the 87 individuals (43%) sampled from the Upper Vienne basin were analysed, which should provide a good estimate of haplotype distribution among these populations. Of these 37 individuals, only three different haplotypes, one already observed in the Allier River in 2011 (Ht26, Persat *et al.*, 2013), and two unknown up to now (Ht24 and Ht25) were revealed (Tab. IV). Among the latter, one is common in all the Vienne populations whereas the second appeared only at low frequencies in the Combade River. None of these haplotypes appeared among the nine haplotypes recorded from the four fish farms. While eight of the nine fish-farm haplotypes grouped with clades not representative of the Loire (i.e. Danube, Rhone and Rhine basins), one newly reported Loire-clade haplotype (Ht23) was found in four individuals of the Besse fish farm.

Based on the median-joining nework (Fig. 3), the Loire haplotypes form a very distinct clade, and this clade is well structured reflecting each of the three main Loire sub-drainages still inhabited by grayling. Thus, the two new “Vienne” haplotypes (Ht24, Ht25) form a distinct branch within the Loire clade, whereas the third “Vienne” haplotype (Ht26) is closely related to the other Allier branch haplotypes and the Loire clade haplotype from the Besse fish farm (Ht23) as well. In summary, at the mtDNA level, there was no evidence of allochthonous introgression into the Upper Vienne basin.

Based on microsatellite data, a number of samples deviated from HWE with positive F_{IS}-values, including the Combade sample, and two of the four fish farms (Besse and Chazey-Bons) suggesting substructure, whereby the Besse fish farm sample additionally had one locus with a significant negative F_{IS}-value (Tab. V). In contrast, the upper Loire sample significantly deviated from HWE with a negative F_{IS}-value suggesting, together with the low allelic diversity, family structure within the sample. The mean number of alleles for Loire basin samples (i.e. Upper Vienne, Upper Loire and Allier) was rather low compared to the other samples, but within the Loire basin, the Vienne reach appeared to be more diverse than the Upper Loire (Tab. V). The highest expected heterozygosity was found in the Besse fish-farm sample, being higher than the most diverse natural population in this study, the Lower Ain River. However, considering the deviation from HWE, this sample is likely composed of more than one source population.

The lack of variation at the Thy1 seems to be specific to the Upper Vienne drainage basin, while the low variability of Thy54 and Tht123, corresponds to their usual low variability elsewhere.

The Factorial Correspondence Analysis based on the presence and absence of alleles within individuals provides an overview of the genetic relationships among all samples (Fig. 4A, B). The first factor strongly discriminates individuals from the Loire basin (Vienne, Upper Loire and Upper Allier) from all others. The second factor especially discriminates Danubian samples from Rhine and Rhone (i.e. Ain, Chazey-Bons, Obenheim), whereas the third factor distinguishes the Ain and Chazey-Bons (both of Rhone origin) from the Obenheim (Rhine origin) samples. Finally, the fourth factor discriminates the Loire samples from each

Table V. - Summary statistics of the 10 microsatellite loci for each population. *N*: individual number of samples; A_N: number of alleles; A_R: allelic range; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: inbreeding. The Upper Vienne sample sites are listed both separately and as one pooled group. Bold numbers with an asterisk indicate a statistically significant deviation from HWE.

	N		*Tar101*	*Tar106*	*Thy1*	*Thy62*	*Tth305*	*Tth447*	*Tar104*	*Thy54*	*Tth414*	*Tth446*	*Overall*
Upper Vienne (Combade)	37	A_N	7	8	1	5	6	4	9	3	7	5	55
		A_R	260-336	191-307	109-109	202-256	274-316	169-179	173-241	191-199	356-502	227-251	
		H_O	0.737	0.583	0	0.568	0.546	0.000	0.733	0.629	0.565	0.639	0.554
		H_E	0.748	0.682	0	0.562	0.632	0.540	0.668	0.613	0.686	0.745	0.217
		F_{IS}	0.042	0.159	*NA*	0.003	0.160	0.132	-0.080	-0.011	0.198	0.156	**0.087***
Upper Vienne (Vienne upstream)	34	A_N	3	6	1	4	6	3	6	3	10	5	47
		A_R	260-284	191-307	109-109	204-256	274-316	169-179	185-241	191-199	356-514	227-251	
		H_O	0.450	0.677	0	0.677	0.552	0.618	0.765	0.647	0.680	0.765	0.583
		H_E	0.405	0.751	0	0.653	0.617	0.629	0.731	0.635	0.770	0.695	0.589
		F_{IS}	-0.086	0.114	*NA*	-0.022	0.123	0.033	-0.032	-0.003	0.137	-0.082	0.026
Upper Vienne (Vienne downstream)	15	A_N	5	6	1	4	4	3	6	3	7	5	44
		A_R	260-336	191-307	109-109	204-256	274-316	169-179	173-241	191-199	356-502	227-251	
		H_O	0.615	0.867	0	0.800	0.692	0.800	0.583	0.357	0.667	0.667	0.605
		H_E	0.701	0.738	0	0.651	0.660	0.640	0.618	0.554	0.821	0.738	0.612
		F_{IS}	0.162	-0.141	*NA*	-0.196	-0.009	-0.217	0.099	0.387	0.244	0.130	0.053
Upper Vienne (Maulde)	2	A_N	1	2	1	1	1	1	2	3	1	2	15
		A_R	276-276	191-231	109-109	256-256	304-304	175-175	185-225	191-199	502-502	239-251	
		H_O	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
		H_E	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
		F_{IS}	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*	*NA*
Upper Vienne (sub-total)	88	A_N	7	9	1	6	7	4	11	4	13	5	67
		A_R	260-336	191-311	109	202-256	274-316	169-179	173-241	191-199	356-514	227-276	
		H_O	0.574	0.667	*NA*	0.636	0.561	0.602	0.718	0.600	0.610	0.690	0.629
		H_E	0.672	0.7544	*NA*	0.647	0.632	0.649	0.737	0.644	0.816	0.767	0.702
		F_{IS}	**0.154***	**0.122***	*NA*	0.023	0.120	0.161	-0.033	0.074	**0.260***	**0.106***	**0.111***
Upper Loire	43	A_N	5	8	3	4	5	4	4	3	5	4	45
		A_{range}	256-348	191-307	109-125	204-264	274-316	169-185	185-225	191-197	350-478	235-243	
		H_O	0.615	0.698	0.372	0.762	0.7692	0.767	0.707	0.721	0.442	0.721	0.657
		H_E	0.649	0.643	0.391	0.717	0.726	0.617	0.583	0.546	0.371	0.623	0.586
		F_{IS}	0.065	-0.074	0.059	-0.051	-0.046	**-0.232***	**-0.202***	**-0.310***	-0.180	-0.146	**-0.109***
Allier	42	A_N	7	7	3	5	5	5	6	4	11	4	57
		A_R	252-296	191-311	109-129	230-260	284-316	169-181	169-241	189-199	356-514	227-243	
		H_O	0.600	0.825	0.683	0.725	0.775	0.590	0.564	0.100	0.821	0.575	0.626
		H_E	0.618	0.793	0.558	0.710	0.738	0.530	0.599	0.120	0.818	0.575	0.606
		F_{IS}	0.042	-0.027	**-0.213***	-0.009	-0.037	-0.033	0.071	0.177	-0.010	0.013	-0.020
Ain	43	A_N	16	10	10	4	14	7	19	11	18	8	117
		A_R	208-324	191-299	113-157	188-208	282-350	160-190	157-285	189-211	366-558	215-259	
		H_O	0.850	0.714	0.781	0.325	0.825	0.275	0.805	0.905	0.691	0.738	0.691
		H_E	0.882	0.765	0.795	0.326	0.777	0.251	0.891	0.845	0.757	0.801	0.709
		F_{IS}	0.049	0.079	0.031	0.016	-0.049	-0.08	**0.109***	-0.058	0.100	0.091	0.038
Besse	29	A_N	7	12	7	9	7	8	10	13	9	6	88
		A_R	204-324	239-283	109-136	202-234	284-382	156-176	149-231	177-231	348-494	219-279	
		H_O	0.593	0.966	0.828	0.857	0.546	0.655	0.552	1.000	0.417	0.370	0.678
		H_E	0.723	0.858	0.823	0.760	0.778	0.725	0.876	0.885	0.789	0.630	0.785
		F_{IS}	**0.198***	-0.108	0.012	-0.110	**0.320***	0.038	**0.386***	**-0.112***	**0.488***	**0.428***	**0.154***

Table V. - Continued.

	N		*Tar101*	*Tar106*	*Thy1*	*Thy62*	*Tth305*	*Tth447*	*Tar104*	*Thy54*	*Tth414*	*Tth446*	*Overall*
Chauvey	33	A_N	12	6	5	7	0	3	7	11	3	0	54
		A_R	264-352	175-283	110-134	214-248	*NA*	152-178	149-239	175-235	348-356	*NA*	
		H_O	0.833	0.781	0.697	0.656	*NA*	0.424	0.394	0.844	0.505	*NA*	0.642
		H_E	0.840	0.709	0.702	0.585	*NA*	0.574	0.346	0.828	0.548	*NA*	0.642
		F_{IS}	0.025	-0.086	0.022	-0.105	*NA*	**0.038***	**-0.124***	-0.004	-0.069	*NA*	-0.002
Obenheim	40	A_N	3	10	4	5	8	7	12	10	12	9	80
		A_R	208-320	187-215	113-145	202-214	366-414	152-196	149-285	181-235	352-526	207-275	
		H_O	0.275	0.950	0.275	0.575	0.889	0.825	0.923	0.790	0.526	0.872	0.690
		H_E	0.247	0.849	0.248	0.660	0.804	0.759	0.802	0.708	0.813	0.843	0.673
		F_{IS}	-0.103	**-0.106***	-0.097	0.141	-0.091	-0.073	**-0.139***	-0.102	**0.364***	-0.022	-0.012
Chazey-Bons	43	A_N	10	13	10	8	0	8	14	10	13	6	92
		A_R	196-316	215-291	110-157	188-230	*NA*	156-188	149-273	177-231	348-538	227-279	
		H_O	0.564	0.875	0.585	0.595	*NA*	0.644	0.884	0.814	0.707	0.368	0.671
		H_E	0.823	0.864	0.806	0.620	*NA*	0.605	0.868	0.770	0.829	0.741	0.769
		F_{IS}	**0.326***	0	**0.285***	0.051	*NA*	-0.073	-0.007	-0.046	**0.159***	**0.512***	**0.151***
Kohlenbach	25	A_N	14	8	5	6	0	4	8	8	4	0	57
		A_R	208-352	195-291	110-140	202-224	*NA*	152-170	149-255	175-229	348-386	*NA*	
		H_O	0.960	0.840	0.708	0.625	*NA*	0.667	0.739	0.826	0.636	*NA*	0.750
		H_E	0.894	0.825	0.626	0.705	*NA*	0.561	0.682	0.771	0.656	*NA*	0.715
		F_{IS}	-0.054	0.002	-0.111	0.134	*NA*	-0.207	-0.106	-0.049	0.053	*NA*	-0.028

Table VI. - Pairwise F_{ST} distances in eight populations. Significant F_{ST} values are indicated in bold letters.

	Upper Loire	Allier	Ain	Besse	Chauvey	Obenheim	Chazey-Bons	Kohlenbach
Upper Vienne	0.261	0.260	0.285	0.243	**0.356**	**0.339**	0.265	**0.320**
Upper Loire		**0.312**	**0.323**	0.265	**0.356**	**0.361**	**0.302**	**0.339**
Allier			**0.308**	0.225	**0.373**	**0.341**	0.283	**0.334**
Ain				0.204	**0.324**	0.264	0.085	0.284
Besse					0.210	0.179	0.136	0.172
Chauvey						**0.342**	0.252	0.119
Obenheim							0.240	0.296
Chazey-Bons								0.209

other, primarily Allier from Upper Loire with the Upper Vienne locations in an intermediate position.

Two series of Structure analyses were carried out, one with 10 loci, and one with the loci Thy1 and Tth305 removed due to their lack of polymorphism in several populations. For the reduced data set, the Evanno approach revealed K = 8 as the most likely number of populations (Fig. 5A). Hereby the various samples sites of the Upper Vienne system appear as one homogeneous group, and the other two sub-drainages of the Loire catchment (Upper Loire and Allier) each appear separately as homogeneous groups. Collectively, these results strongly support the absence of non-Loire genotypes throughout the Loire catchment. Two additional genetic groups are clearly revealed representing the Obenheim hatchery, and the Danubian Kohlenbach population together with the Chauvey-bons hatchery confirming the notion that this hatchery stock was founded with Danube drainage grayling. Additional genetic groups were identified in the samples from Chazey-Bons and Besse hatcheries but these populations are clearly made up of multiple genotype groups. Finally, the Ain population, while revealing an additional genetic subdivision in this Structure analysis, shows admixture from genotypes apparently common to those in the Chazey-Bons hatchery. Re-running the structure with all 10 loci merely shows additional resolution with the genetic subdivisions of the Besse and Chazey-Bons hatcheries with no effect on the genetic composition or inferences concerning the Loire catchment (Fig. 5B). Overall, moderate to high F_{ST}-values for nearly all pairwise comparisons (Tab. VI) also supported the genetic differences seen among populations in the Structure analysis.

DISCUSSION

The genetic analysis of four European grayling populations from the Upper Vienne River drainage basin using both nDNA (microsatellites) and mtDNA markers shows that they all share a typical Loire (ligerian) genetic profile, but with sufficient differentiation from other sub-drainages to strongly support that they represent pure or nearly pure native stocks. Despite decades of stocking, much of which likely implied foreign strains of grayling, no sign of introgression from foreign sources was detected. The occurrence of a shared haplotype between the lowermost population in the Vienne River and fish from the Allier River is likely based on human-induced exchanges within the Loire catchment (e.g. from the Besse farm), but could also be explained by historical exchange or retained ancestral polymorphisms. We note that these river stretches are separated by at least 1000 km of river, most of which is heavily dammed so that gene exchange in modern times is virtually impossible. While the above-noted haplotype was not found in our sample from the Besse population, the sample from this hatchery need not be representative of the whole production of the facility. The Besse hatchery lineage has a long and complex history stemming back to the early rearing attempts at the CSP fish farm of Augerolles (Carmié *et al.,* 1985; Persat *et al.*, 1997), which mainly started with imported eggs before shifting to local spawners from the Loire basin. Our current data set, however, is likely insufficient to evaluate quantitatively the lineage composition of the Besse hatchery. Nonetheless, it is clear that the current sample from the Besse hatchery reveals at least two genotypic groups that do not appear in any sample from the Loire catchment. Likewise, none of the other three hatcheries, each of which is genetically distinct, appears to have contributed to the current genetic architecture of the upper Loire.

The distinct genotypic group in the Structure analysis combined with the mtDNA haplotype data strongly support that the Obenheim hatchery consists of Rhine basin grayling, and this confirms the report that the strain originated from wild-caught spawners from the Steina brook, a tributary of the Wutach River in Baden-Württemberg (Martin Gerber, pers. com.). Thus, we have highly recognizable genotypes (microsatellites) and haplotypes (mtDNA) from three distinct basins (Rhone, Rhine and Danube) from outside of the Loire catchment.

There is less than 100 km of grayling habitat currently in the Upper Vienna region (Fig. 1) and thus stocking densities roughly reached up to > 1000 individuals per km in some years (Tab. I), for the entire region. Densities of wild fish are not available, but are considered low. Overall, the most likely explanation for the lack of a genetic trace for the largely non-native strains of stocked fish is very low survival after release.

The mechanisms responsible for the low survival are not evaluated in this study, but more than likely reflect the typical suite of factors revealed for other salmonid species. That is, a combination of maladaptation (genetic) for the given environment, behavioural or physiological imprinting in captivity (e.g. adaption to artificial diet, still versus running water), and poor competitiveness with the existing native stocks. Some observations on the Rhone River by-passed section in Chautagne (Savoy; Persat *et al.*, 1997) shown that of 54 000 fingerlings stocked in late August 1993, an unknown proportion was still present three months later despite of a severe period of high water levels soon after having being released. However, they were much smaller sized (9.8 cm versus 14.9 cm) and occupied a much more quiet habitat (shallows at 10 cm depth with a rather low water velocity) along the gravel shore compared to typical habitat of the resident native grayling that are found in the main riffles of the river at 40-80 cm depth (Mallet *et al.,* 2000). From our study on the Rhone in Chautagne, we assume that the stocked grayling occupied sub-optimal to poor habitat and thus were marginalized and experienced very poor survival (no trace of survival of the fishes was found in the years thereafter).

While from a conservation standpoint it is positive that genetic introgression has not taken place, we should recognize that this does not exclude the possibility that continuous stocking has had negative consequences in raising short-term intraspecific competition, introducing pathogens, promoting higher densities of predators, and elevating angler expectations.

Ongoing investigations on other grayling populations in France seem to confirm that the competitive advantage of native stocks over translocated ones is often the rule; observed examples of successful stocking programs are mainly found in rivers free of native populations such as in the Seine and Garonne basins (Persat, 2011). However, wide-scale contact and interbreeding is reported between close lineages in the upper Rhone and Ain river basins where Rhone related populations more or less mix with genotypes of Swiss-Rhine origin, especially those found in the Geneva Lake drainage basin (Cattaneo *et al.,* 2011). Both Cattaneo *et al.* (2011) and Vonlanthen and Salzburger (2011), with a more detailed sampling, also clearly demonstrated the survival of stocked Rhone basin grayling in the Birs River of the Rhine catchment in Switzerland, though the river is severely fragmented by dams, and not all stretches demonstrated the sympatry of native and stocked lineages. In contrast, both Cattaneo *et al.* (2011) and Vonlanthen and Schlunke (2015) reported no genetic trace of multiple stockings from multiple sources (for the latter study, three hatcheries were documented) throughout the Aar, Limmat and Reuss rivers in the Rhine catchment of Switzerland. For the latter study, the documented stocked fish were of the same major lineage (Rhine catchment), but nonetheless clearly distinguish-able with microsatellite markers. Stocking densities ranged from 30 000 to over 120 000 per year for 18 years, across river reaches of ca. 100 km. Yet, as it was the case in our study, no trace of the hatchery material was found. Thus despite knowledge that stocked grayling of various divergent origins do introgress or even in some situations replace native stocks (e.g. Meraner *et al.,* 2014; Weiss *et al*., 2015) our study and the cited reports provide multiple examples of complete failure of stocked lineages to survive and introgress into native populations. Currently, we lack insight into the decisive mechanisms that support or hinder survival and introgression of stocked grayling. Nonetheless, a frequently cited factor for the promotion of hybridization and introgression, and one that also has at least some supportive data for salmonid fishes is the environmental conditions and demographic health of the native populations (Rhymer and Simberloff, 1996; Seehausen *et al.,* 2008; Bittner *et al.,* 2010; Winkler *et al.,* 2011). That is, the less disturbed systems with healthy native populations present a more likely barrier to introgression than disturbed environments with low population density.

The efficacy of management focused on stocking hatchery grayling is thus called into question, especially for flowing water habitats that contain native stocks. This once again calls for alternative investment in improved habitat conditions, including restoration of the riverbed and connectivity, reduction of hydropeaking, increase of residual flows and improved monitoring and control of reservoir flushing operations.

Finally, if a stocking appears definitely necessary in a reach where the grayling is obviously extinct, it should be based on translocation of wild fry as young as possible (such as at emergence) because Bardonnet and Gaudin (1990) showed that even the artificial conditions during the incubation could alter the behaviour and emergence timing of the larvae. Of course, these fry should be collected in the same catchment, and as close as possible to the reach to stock in order to have the highest likelihood of introducing genotypes that are adapted to the local environmental conditions. Another promising approach that is gaining popularity in central Europe is referred to as “cocooning”. The approach is based on an adaptation of so-called Vibert or Whitlock-Vibert breeding boxes (Vibert, 1953; Whitlock, 1978), whereby several changes to these designs as well as the general application strategy have proved to be very successful for several salmonid species including grayling (Holzer *et al.,* 2011). The overall concept supports the maximal degree of adaptation and conditioning to the local environment as the fish are hatched in the riverbed itself with the only artificial component of the procedure being the choice and stripping of parental fish.

Acknowledgements. – We are deeply indebted to the “Fédération Nationale de la Pêche en France” for having funded half the costs of this work, with the remaining support stemming from our institutions. We are also indebted to two Angling Societies for having kindly provided samples of the fish they stocked, and to Martin Gerber for those of the Federal 67 Obenheim fish farm. We also thanks the fisheries officers of the Haute-Vienne Fishing Federation for their help in the electric fishing operations performed to collect the wild specimens used in this study. Finally, we are also very grateful to two anonymous referees who greatly helped us to improve this paper with their useful and accurate criticisms and comments.

REFERENCES

BARDONNET A. & GAUDIN P., 1990. - Influence de la lumière au cours de l’ontogenèse sur l’expression du rythme d’émergence de l’alevin d’ombre commun *Thymallus thymallus* (L., 1758). *Bull. Fr. Pêche Piscic*., 317: 35-49.

BELKHIR K., BORSA P., CHIKHI L., RAUFASTE, N. & BONHOMME F., 1996-2004. - GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Univ. Montpellier II, France.

BITTNER D., EXCOFFIER L. & LARGIADÉR C.R., 2010. - Patterns of morphological changes and hybridization between sympatric whitefish morphs (*Coregonus* spp.) in a Swiss lake: a role for eutrophication? *Mol. Ecol*., 19: 2152-2167.

CATTANEO F., WINKLER K., GRIMARDIAS D., PERSAT H. & WEISS S., 2011. - Caractérisation génétique des populations d’ombre commun (*Thymallus thymallus* L.) de Suisse et France transfrontalière, Applications à la conservation et a la gestion de l’espèce. 76 p. Hesso Genève (Haute École spécialisée de Suisse occidentale).

CARLSTEIN M., 1997. - Effects of rearing technique and fish size on post-stocking feeding, growth and survival of European grayling, *Thymallus thymallus* (L.). *Fish. Manage. Ecol.,* 4: 391-404.

CARMIÉ H., MORELET B., MAISSE G., JONARD L. & CUINAT R., 1985. - Observations sur la reproduction artificielle de l’ombre commun (*Thymallus thymallus*). *Bull. Fr. Pêche Piscic.,* 296: 2-16.

CHAMPIGNEULLE A., DEGIORGI F., RAYMOND J.C. & CACHERA S., 2002. - Dynamique temporelle de la contribution du repeuplement en stades précoces de truite (*Salmo trutta* L.) dans la population en place et dans la pêche sur le Doubs franco-suisse. *Bull. Fr. Pêche Piscic*., 365/366: 471-485.

CRESSWELL R.C., 1981. - Post-stocking movements and recapture of hatchery-reared trout into flowing waters — a review. *J. Fish Biol.,* 18: 429-442.

EARL D.A. & VONHOLDT B.M., 2012. - STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Res*., 4(2): 359-361.

EVANNO G., REGNAUT S. & GOUDET J., 2005. - Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.,* 14: 2611-2620.

FALUSH D., STEPHENS M. & PRITCHARD J.K., 2003. - Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics,* 164: 1567-1587.

FINNEGAN A.K. & STEVENS J.R., 2008. - Assessing the long-term genetic impact of historical stocking events on contemporary populations of Atlantic salmon (*Salmo salar* L.). *Fish. Manage. Ecol*., 15: 315-326.

GROSS R., KÜHN R., BAARS M., SCHRÖDER W., STEIN H. & ROTTMANN O., 2001. - Genetic differentiation of European grayling populations across the Main, Danube and Elbe drainages in Bavaria. *J. Fish Biol.,* 58: 264-280.

GUM B., GROSS R. & KUEHN R., 2005. - Mitochondrial and nuclear DNA phylogeography of European grayling (*Thymallus thymallus*): evidence for secondary contact zones in central Europe. *Mol. Ecol.,* 14: 1707-1725.

GUM B., GROSS R. & GEIST J., 2009. - Conservation genetics and management implications for European grayling, *Thymallus thymallus*: synthesis of phylogeography and population genetics. *Fish. Manage. Ecol.*, 16: 37-51.

HANSEN M.M., FRASER D.J., MEIER K. & MENSBERG K.L.D., 2009. - Sixty years of anthropogenic pressure: a spatio-temporal genetic analysis of brown trout populations subject to stocking and population declines. *Mol. Ecol.*, 18: 2549-2562.

HARBICHT A., WILSON C.C. & FRASER D.J., 2014. - Does human-induced hybridization have long-term genetic effects? Empirical testing with domesticated, wild and hybridized fish populations. *Evol. Appl.,* 7: 1180-1191.

HOLZER G., UNFER G. & HINTERHOFER M., 2011. - “Coocooning” eine alternative Methode zur fischereilichen Bewirtschaftung. *Österreichs Fisch.,* 64: 16-27.

HUET M., 1949. - Aperçu des relations entre la pente et les populations piscicoles des eaux courantes. *Rev. Suisse Hydrol.*, 9: 332-351.

LARGIADÈR C.R. & SCHOLL A., 1996. - Genetic introgression between native and introduced brown trout *Salmo trutta* L. populations in the Rhône River Basin. *Mol. Ecol.*, 5: 417-426.

MALLET J.-P., LAMOUROUX N., SAGNES P. & PERSAT H., 2000. - Habitat preferences of European grayling in a medium size stream, the Ain River, France. *J. Fish Biol.*, 56: 1312-1326.

MARIE A.D., BERNATCHEZ L. & GARANT D., 2010. - Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*). *Mol. Ecol.*, 19: 2025-2037.

MERANER A., UNFER G. & GANDOLFI A., 2013. - Good news for conservation: limited genetic signatures of inter-basin fish transfer in *Thymallus thymallus* (Salmonidae) from the Upper Drava River. *Knowl. Manag. Aquat. Ecosyst.,* 409: 1-17.

MERANER A., CORNETTI L. & GANDOLFI A., 2014. - Defining conservation units in a stocking-induced genetic melting pot: unraveling native and multiple exotic genetic imprints of recent and historical secondary contact in Adriatic grayling. *Ecol. Evol.,* 4(8): 1313-1327.

MILLER R.B., 1953. - Comparative survival of wild and hatchery-reared cutthroat trout in a stream. *Trans. Am. Fish. Soc.,* 83: 120-130.

MILLER R.B., 1958. - The role of competition in the mortality of hatchery trout. *J. Fish. Res. Bd Can.,* 15: 27-45.

MILLER S.A., DYKES D.D. & POLESKY H.F., 1988. - A simple salting out procedure from human nucleated cells. *Nucleic Acids Res.,* 16: 1215.

NÄSLUND I., 1998. - Survival and dispersal of hatchery-reared brown trout, *Salmo trutta*, released in small streams. *In*: Stocking and Introduction of Fish (Cowx I.G., ed.), pp. 59-76. Oxford: Fishing News Books, Blackwell Science.

PERSAT H., 1996. - Threatened populations and conservation of the European grayling *Thymallus thymallus* (L., 1758). *In*: Conservation of Endangered Freshwater Fish in Europe (Kirchhofer A. & Hefti D., eds), pp. 233-247. Basel: Birkhäuser Verlag.

PERSAT H., 2011. - L’ombre commun *Thymallus thymallus* (Linnaeus, 1758). *In*: Les Poissons d’eau douce de France (Keith P., Persat H., Feunteun E. & Allardi J., eds), pp. 413-415. Mèze: Biotope; Paris: MNHN.

PERSAT H. & EPPE R., 1997. - Alevinage, pollution et cloisonnement de l’espace fluvial dans les structures génétiques des populations de poisson: l’ombre commun, *Thymallus thymallus*, dans le Rhône au niveau de la Savoie. *Bull. fr. Pêche Piscic.*, 70(344-45): 287-299.

PERSAT H., PATTEE E. & ROUX A.L., 1978. - Origine et caractéristiques de la distribution de l’ombre commun, *Thymallus thymallus* (L. 1758) en Europe et en France. *Verh. Int. Verein. Limnol.*, 29: 217-2121.

PERSAT H., EPPE R. & MALLET J.-P., 1997. - Conséquences démographiques et génétiques des alevinages sur la population d’0mbre commun du Rhône Savoyard. 47 p. Technical report, Convention CSP n° 93191, UMR 5023, Univ. Lyon I.

PERSAT H., MATTERSDORFER K., CHARLAT S., SCHENEKAR T. & WEISS S., 2013. - Diversité génétique des populations d’ombre commun du département de la Haute-Vienne. 30 p. Technical Report, UMR 5023 Univ. Lyon 1, and Institute für Zoologie Univ. Graz.

PRITCHARD J.K., STEPHENS M. & DONNELLY P., 2000. - Inference of population structure using multilocus genotype data. *Genetics*, 155: 945-959.

RHYMER J.M. & SIMBERLOFF D., 1996. - Extinction by hybridization and introgression. *Ann. Rev. Ecol. Syst.*, 27: 83-109.

SEEHAUSEN O., TAKIMOTO G., ROY D. & JOKELA J., 2008. - Speciation reversal and biodiversity dynamics with hybridization in changing environments. *Mol. Ecol.*, 17: 30-44.

SUŠNIK S., SNOJ A. & DOVC P., 1999. - Microsatellites in grayling (*Thymallus thymallus*): comparison of two geographically remote populations from the Danubian and Adriatic river basin in Slovenia. *Mol. Ecol.*, 8: 1756-1758.

SUŠNIK S., KNIZHIN I., SNOJ A. & WEISS S., 2006. - Genetic and morphological characterization of a Lake Ohrid endemic, *Salmo* (*Acantholingua*) *ohridanus* with a comparison to sympatric *Salmo trutta*. *J. Fish Biol.,* 68(A): 2-23.

TAMURA K., DUDLEY J., NEI M. & KUMAR S., 2007. - MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.*, 24: 1596-1599.

TUREK J., RANDÁK T, HORKÝ P., ZLÁBEK V., VELÍŠEK J., SLAVÍK O. & HANÁK R., 2010. - Post-release growth and dispersal of pond and hatchery-reared European grayling *Thymallus thymallus* compared with their wild conspecifics in a small stream. *J. Fish Biol.,* 76: 684-693.

UIBLEIN F., JAGSCH A., HONSIG-ERLENBURG W. & WEISS S., 2001. - Status, habitat use, and vulnerability of the European grayling in Austrian waters. *J. Fish Biol.*, 59(sA): 223-247.

VIBERT R., 1953. - Plastic hatching box for stocking trout and salmon. *Prog. Fish Cult.,* 13.

VINCENT E.R., 1987. - Effects of stocking catchable-size hatchery rainbow trout on two wild trout species in the Madison River and O’Dell Creek, Montana. *N. Am. J. Fish. Manage.,* 7: 91-105.

VONLANTHEN P. & SALZBURGER W., 2011. - Populationsgenetische Untersuchungen der Äschen in der Birs. Endbericht im Auftrag der Kantonaler Fischereiverband Baselland. 14 p. Switzerland: Kantonaler Fischereiverband Baselland (KFVBL).

VONLANTHEN P. & SCHLUNKE D., 2015. - Erfolgskontrolle Besatzmassnahmen und Populationsgenetische Untersuchungen der Äschen im Kanton Aargau. 33 p. Aquabios GmgH, Auftraggeber: Departement Bau, Verkerkehr und Umwelt, Sektion Jagd und Fischerei, Kanton Aargau.

WEISS S. & SCHMUTZ S., 1999. - Performance of hatchery-reared brown trout and their effects on wild fish in two small Austrian streams. *Trans Am. Fish. Soc.*, 128: 302-316.

WEISS S., PERSAT H., EPPE R., SCHLÖTTERER C. & UIBLEIN F., 2002. - Complex pattern of colonization and refugia revealed for European grayling *Thymallus thymallus*, based on complete sequencing of the mitochondrial DNA control region. *Mol. Ecol.*, 11: 1393-1407.

WEISS S., KOPUN T. & SUŜNIK BAJEC S., 2013. - Assessing natural and disturbed population structure in European grayling, *Thymallus thymallus* (Salmonidae): melding phylogeographic, population genetic, and jurisdictional perspectives for conservation planning. *J. Fish Biol.,* 82: 505-521.

WEISS S., MATTERSDORFER K. & BÖRGER C., 2015. - Genotypisierung, nachhaltige Sicherung und Bewirtschaftung regionaler Äschenbesände in anthropogen veränderten Gewässersystemen (Alpäsch) – Subauftrag – Genetische Charakterisierung – Endbericht. Interreg IV Italien-Österreich, Projekt „Alpäsch“ (6142) CUP H21J12000040007, Im Auftrag von Autonome Provinz Bozen-Südtirol, Abteilung 33, Land-und Forstwirtschaftliches Versuchswesen, Laimburg 6 Pfatten, I-39040.

WHITLOCK D., 1978. - The Whitlock-Vibert Box Handbook. 47 p. Federation of Fly Fishers [cited in Holzer *et al.*, 2011].

WINKLER K.A, PAMMINGER-LAHNSTEINER B., WANZENBÖCK J. & WEISS S., 2011. - Hybridization and restricted gene flow between native and introduced stocks of Alpine whitefish (*Coregonus* sp.) across multiple environments. *Mol. Ecol.*, 20: 456-472.